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# The Effects of Diflubenzuron on a Complex Laboratory Stream Community

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16. ABSTRACT

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Archives of Environmental Contamination and Toxicology

# The Effects of Diflubenzuron on a Complex Laboratory Stream Community

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**Abstract.** Effects were assessed on the total biological community in complex laboratory streams caused by continuous exposure to diflubenzuron (1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea). The insect fauna suffered direct toxic effects at concentrations of 1.0 μg/L and greater. The algal and fungal floras were mildly affected at the same concentrations; apparently indirect effects in response to the reduced herbivore and shredder components of the insect fauna. No effects were observed on the bacteria, oligochaetes or gastropods, at any of the test concentrations. Within the insect fauna, differences in sensitivities were observed: mayflies and stoneflies were affected at 1.0 μg/L, dipterans were affected at 10.0 μg/L, and coleopterans were apparently unaffected at any of the test concentrations. Effects on caddisflies could not be determined due to their failure to become established in the stream systems. These differences between insect orders in sensitivity, coupled with differences in generation time, may reduce the overall benefits of the insecticide. For example, if diflubenzuron is used for chironomid control, the result may be more severe and longer lasting effects on nontarget mayfly and stonefly populations.

The insect growth regulator, diflubenzuron (1-(4-chlorophenyl)-3(2,6-difluorobenzoyl)-urea) (Dimilin®), is a highly regarded compound for the control of agricultural and forest pest insect populations because of its specificity to insects, low degree of bioaccumulation, and rapid rate of environmental degradation. Its specificity is due to a unique mode of toxicity—the blockage of chitin synthesis (Post *et al.* 1974; Hajjar and Casida 1978; Meola and Mayer 1980)—which should limit its direct toxic influence to organisms with chitinous exoskeletons. The available laboratory toxicity tests have supported this hypothesis; among the species studied, only arthropods were sensitive, while other phyla of plants and animals were unaffected (Mulla *et al.* 1974; Mulla and Darwazeh 1976; Miller *et al.* 1974; Miller 1974; Moore and Tuft 1975; Wright and Harris 1976; Wright and Spates 1976; Lacey and Mulla 1977; Ivie and Wright 1978; Julin and Sanders 1978; Lacey and Mulla 1978a and b; McKague and Pridmore 1978; Nimmo *et al.* 1979 and 1980). Relatively low levels of bioconcentration, less than 100× in fish and 1000× in insects (Metcalf *et al.* 1975; Schaefer *et al.* 1979), are reportedly due to a comparatively high water solubility (Metcalf *et al.* 1975; Ivie *et al.* 1980). Decomposition of diflubenzuron in water is mediated via biological and physical processes; the rate is increased by high temperature and pH (Ruzo *et al.* 1974; Schaeffer and Dupras 1976 and 1977; Ivie *et al.* 1980; Pritchard and Bourquin 1979).

Before the use of Dimilin® is greatly expanded, further assessment of its actual specificity in natural communities is necessary, which can be estimated from an analysis of impacts on complex systems rather than on single species populations. Complex systems contain many interacting species, representing several tropic levels. The relative sensitivities of many target and non-target species can be determined, incorporating both direct and indirect toxic effects. Single species toxicity tests are limited to a few culturable species. Therefore, relative sensitivities of most non-target species cannot be determined and only direct toxic effects can be assessed.

Several aquatic field studies have elucidated the specificity of diflubenzuron (Miura and Takaheshi 1975; Mulla and Darwazeh 1975; Steelman *et al.* 1975; Mulla *et al.* 1976; Ali and Mulla 1977a and b, 1978a and b; Colwell and Schaeffer 1980). These studies were primarily concerned with effects on the invertebrate fauna and indicated that non-target

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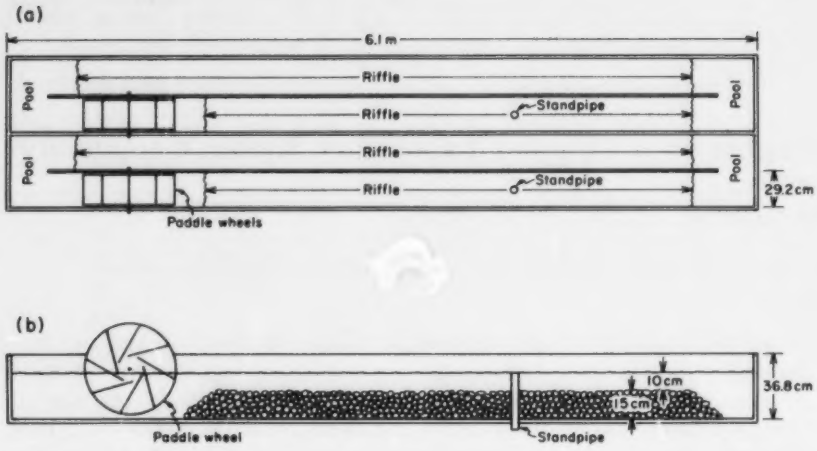


**Fig. 1.** Laboratory stream channels: (a) an overview of a pair of channels (b) a side view of a single channel

species (*i.e.*, mayflies, stoneflies, and copepods) were impacted more severely and required longer recovery periods than the target species (*i.e.*, midges, mosquitos, blackflies). However, no assessment was made of the direct and indirect effects on other taxonomic groups present in the communities.

In this study, the impact of diflubenzuron on several components of a complex stream community was investigated. Replicated laboratory stream communities were established, equilibrated, and subsequently perturbed with diflubenzuron. The invertebrate, algal, fungal, and bacterial components were monitored and alterations in density and/or composition were identified. Analysis of sensitivities of different taxonomic groups was used to establish the degree of specificity of diflubenzuron in the stream systems.

## Materials and Methods

### *Physical Description*

An eight month study (September 1979–May 1980), was made at Western Fish Toxicology Station, part of the Corvallis Environmental Research Laboratory, U.S. Environmental Protection Agency, Corvallis, OR. The experimental system consisted of ten indoor continuous-flow laboratory stream channels. Each channel was a continuous loop (Figure 1) (12.2 m long × 29.2 cm wide × 36.8 cm deep), constructed of plywood and covered with three coats of white enamel epoxy paint (Parker #921, Tacoma, WA). The riffle portions of the channels were uniformly filled with approximately 15 cm of rocks, 7.5–13.0 cm in diameter; the pools remained unfilled. Well water was continually added to the channels at a rate of 5 L/min and maintained at a depth of approximately 10 cm above the rocks, by use of standpipes (see Samuelson 1976 for water quality information). There was a 95% replacement rate of approximately 4.5 hr for the 500 L water volume in each channel. A current of 30 cm/sec was maintained by use of stainless steel motor-driven paddle wheels. A uniform light intensity, approximately 200 ft candles, was maintained over each channel with suspended metal halide lamps (Maxi-Gro, 1000 watt, Sylvania GTE Agronomics, Waltham, MA)[1]. A naturally varying diurnal light cycle was used; incorporating thirty min dawn and dusk low light periods.

### *Biological Loading*

The biological fauna were introduced into the stream channels naturally through the replacement water supply and artificially through field collections. Introductions via the water inflow were continuous, but limited to micro-organisms (*i.e.*, primarily, algae and bacteria). Periodic field collections supplied more diverse fauna. From August to October, biological collections were made twice weekly from Rock Creek, a second order forest stream in the Mary's River Watershed, 10 miles west of Corvallis. A combination of kicking and brushing techniques dislodged organisms and facilitated net collections. After removing fish and crayfish, the field samples were randomly distributed among the ten stream channels. Detrital material, which was necessary as a food source for the stream decomposers, was manually introduced into the channels. In the field collections, large amounts of leafy and woody material were also trapped. This detritus was distributed among the channels. After initial stocking, monthly supplements of 50 g of freezer-preserved alder leaves were added to each channel.

### *Treatments*

In all ten stream channels, the biological fauna were developed and equilibrated for three months, September to November. The channels were subsequently divided into five pairs and diflubenzuron treatments were applied for the next five months, December to May. Four stream pairs received continuous addition of Dimilin®; each pair receiving one concentration of either 0.1, 1.0, 10, or 50 $\mu$g/l. The diflubenzuron was dissolved in dimethylformamide (DMF) and was introduced into the inflow water via syringe injector pumps (Sage Pump Model 352, Orion Research,

Cambridge, MA); the carrier concentration was maintained at 1 mg/L in all treatment levels. The fifth stream pair served as carrier controls; receiving 1 mg/L of DMF throughout the treatment period.

### *Biological Analyses*

Biological fauna in each stream channel were estimated monthly (October through May). Three samples were taken from each channel; each sample was collected by sealing off a .052 $m^2$ rectangular area with watertight boards, dislodging attached organisms, and siphoning out the water and the suspended organisms. Portions of these samples were then used for invertebrate, algal, bacterial and fungal analyses. The density and composition of the invertebrate fauna of each stream channel were estimated from filtered collections of organisms. One half of each stream sample was passed through a Tyler Equivalent 115 mesh metal screen. The collected organisms were picked from the detritus, identified, and measured. Insects other than the dipteran family Chironomidae were identified to at least the genus level. The Chironomidae were identified to subfamily or tribe.

An estimate of algal biomass was made from the determination of the chlorophyll-a,b,c and phaeophytin concentrations in each stream channel. Each sample was blended, treated with $MgCO_3$, and centrifuged. The precipitate was dissolved in 90% acetone, sonicated, and centrifuged. The resulting supernatant was analyzed spectrometrically at 750, 663, 645, and 630 nm. A trichromatic procedure was used to differentiate between chlorophyll a, b, and c. A monochromatic acid addition procedure was used for phaeophytin determinations (Weber 1973). An estimate of algal cell density in each stream was made from direct cell counts, with a Sedgwick-Rafter counting chamber. Each sample was blended, placed in a chamber, allowed to settle, and counted. The number of algal cells per unit area of stream channel was calculated.

The composition of the diatom flora in each stream channel was estimated from slide preparations. Each sample was blended and an aliquot evaporated onto a glass slide. The organic matter was burned off and the remaining silica valves were mounted in Hyrax. Microscopic identification (1000×, oil immersion) of the first 100 randomly encountered individuals were used to estimate species abundances in each channel (Hustedt 1930; Patrick and Reimer 1966 and 1975).

The density and composition of the bacterial flora in each stream channel were estimated from a sequence of dilution plating, morphological sorting, and classification testing. A 1.0 ml blended aliquot from each stream sample was serially diluted, plated on PYG media and incubated for 48 hr at 20°C. Colonies were counted and typed according to morphological characteristics. Each type was identified to genus by use of dichotomous testing procedures (Buchanan and Gibbons 1974; Shayegani *et al.* 1976; Steele 1979).

The relative density of the fungal flora in each stream channel was estimated from measurements of hyphal abundance on leaf material. A 5.0 gm alder leaf pack was prepared, incubated for six weeks in the channel, removed and cut into 0.5 cm discs. Ten discs were randomly selected, clarified with 5% KOH, hardened with 50% ethanol, and stained with lactophenol-trypan blue. The percent cover of hyphae on the leaf surface was estimated with an ocular micrometer. The composition of the fungal flora in each stream channel was estimated from an analysis of spores on the leaf packs. Twenty leaf discs were incubated overnight in filter-sterilized water to stimulate spore formation. The discs were fixed in lactophenol. Microscopic identification (250×-phase contrast) of spores on the periphery of each disc estimated species richness (Petersen 1962, 1963a and b; Ingold 1942; Ingold and Cox 1957; Ranzoni 1953). Relative species abundances could not be accurately estimated, because of the unknown correlation between spore numbers and hyphal biomass.

### *Chemical Analyses*

The chemical characteristics of the water in the stream channels were monitored throughout the study. Water temperature was measured continuously; pH, DO, hardness and alkalinity weekly; and monthly measurements were taken of nitrate, nitrite, ammonia, organic nitrogen, phosphate, and orthophosphate.

Diflubenzuron concentrations in the water of stream channels at concentrations $\geq 1.0$ $\mu$g/L were monitored weekly throughout the treatment period. Analyses were performed with a single band 254 nm UV detector, a micro-Bondapak C-18 column, and a 60:40 acetonitrile-water mobile phase. The sensitivity limit was 1.0 $\mu$g/L. Monitoring the 0.1 $\mu$g/L concentration was not possible; therefore, nominal values were used.

### *Statistical Analyses*

Between-stream comparisons were made throughout the study period. The parameters of analysis were density and species diversity of invertebrates, biomass, and species richness of algae, density, and genera diversity of bacteria, biomass and species diversity of fungi. Shannon's Index was used for diversity calculations. Total number of taxa was used to represent species richness. The equivalence of the ten stream channels was assessed during the equilibration period. An analysis of variance and, subsequent, pairwise Student–Neuman-Keuls multiple range tests were made for each test parameter. During the treatment period, the existence of differences between each of the four treatment levels and the controls were assessed. A Williams' test (Williams 1972) was performed, using average stream values for each of the two replicates per treatment level, for each test parameter.

## Results

The taxonomic groups comprising the invertebrate fauna exhibited a range of responses to diflubenzuron addition (Table 1). Generally, the insects numbers (Figure 2) and diversity (Figure 3) decreased, the gastropods remained unaffected (Figure 4), and the oligochaetes increased (Figure 5). Within the insect class, reductions in density and diversity were highest at diflubenzuron concentrations of 10 $\mu$g/L and greater; 1.0 $\mu$g/L caused less severe impacts. Reductions in diversity were statistically significant throughout the treatment period. Reductions in density were statistically significant for all but one treatment period. These reductions in density and diversity were apparently due to direct death of organisms. In the affected treatment streams, there was no increased drift of organisms, but there was evidence of abnormal metamorphosis. Within the predominant insect class, general

**Table 1.** Effect of diflubenzuron on stream invertebrates

| | Diflubenzuron Concentration ($\mu$g/L) | Effect |
|---|---|---|
| Ephemeroptera | | |
| *Baetis spp.* | 1.0 | elimination in 1 mo. |
| *Epeoris sg. iron* | 10.0 | elimination in 1 mo. |
| *Epeoris sg. ironodes* | 10.0 | elimination in 1 mo. |
| *Rithrogena spp.* | 1.0 | elimination in 1 mo. |
| *Paraleptophlebia spp.* | 1.0 | elimination in 1 mo. |
| *Ephemerella sg. serratella* | 1.0 | elimination in 1 mo. |
| *Ephemerella sg. drunella* | 1.0 | elimination in 1 mo. |
| Plecoptera | | |
| *Peltoperla spp.* | 1.0 | elimination in 1 mo. |
| *Pteronarcys spp.* | 1.0 | elimination in 1 mo. |
| *Nemoura zapada* | 1.0 | elimination in 1 mo. |
| *Acroneuria californica* | 1.0 | reduction in 3 mo (~80-90%)<br>elimination in 4 mo. |
| *Hesperoperla spp.* | 1.0 | reduction in 3 mo. (~50%)<br>elimination in 5 mo. |
| *Isoperla spp.* | 1.0 | elimination in 1 mo. |
| *Alloperla spp.* | 1.0 | reduction in 1 mo. (~60%)<br>elimination in 3 mo. |
| Diptera | | |
| Chironomini | 10.0 | reduction in 1-4 mo. (10-75%)<br>elimination in 5 mo. |
| Tanytarsini | 10.0 | reduction in 1-4 mo.<br>elimination in 5 mo. |
| Tanypodinae | 10.0 | reduction in 2 mo.<br>elimination in 5 mo. |
| Orthocladiinae | 10.0 | reduction in 1-4 mo.<br>elimination in 5 mo. |
| | 1.0 | reduction in 2-5 mo. |
| Tricoptera | | |
| *Phyacophila spp.* | — | no effect determination possible |
| *Hydropsyche* spp. | — | no effect determination possible |
| *Microsema spp.* | — | no effect determination possible |
| Coleptera-Elmidae | | |
| *Heterlimnius spp.* | — | no effect |
| *Optioservus spp.* | — | no effect |
| *Zaitzevia spp.* | — | no effect |
| *Narpus spp.* | — | no effect |
| Oligochaeta | 10.0 | increase in 2-3 mo. (~200%)<br>reduction in 4 mo. (~95%) |
| Gastropoda | | |
| *Juga plicifera* | — | no effect |

differences in sensitivity between orders were apparent. The mayflies were the most sensitive (Figure 6); they were rapidly and severely impacted at 1 $\mu$g/L, or greater, of diflubenzuron. The stoneflies were next in sensitivity (Figure 7); they were also severely impacted by 1 $\mu$g/L, but survived longer treatment periods. The chironomids were intermediate in sensitivity (Figure 8); they were immediately reduced by 10 $\mu$g/L, but survived at low densities for five months before elimination. The riffle beetles (Figure 9) were apparently the least sensitive, with no noticeable effects at any of the treatment concentrations. It was very difficult to determine the sensitivity of the caddisflies (Figure 10), because both the control and treatment populations disappeared rapidly.

The algae in the stream communities experienced increased biomass associated with the addition of 1 $\mu$g/L or more of diflubenzuron. Direct counts of algal cells were elevated in the treatment streams throughout the treatment period with statistically significant differences during the last two months (Figure 11). Concentrations of chlorophylls a and c and phaeophytin were also elevated in the treatment streams throughout the period with statistically significant differences during the first, third, and fourth months (Figure 12a–c). Shifts in the algal community structure were observed in association

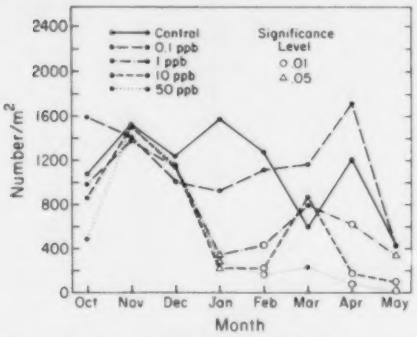


**Fig. 2.** Density of the total insect fauna for each treatment level as a function of time

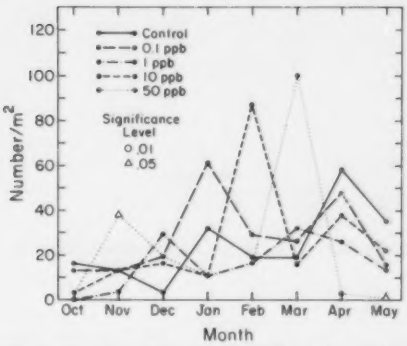


**Fig. 4.** Density of the gastropod fauna for each treatment level as a function of time

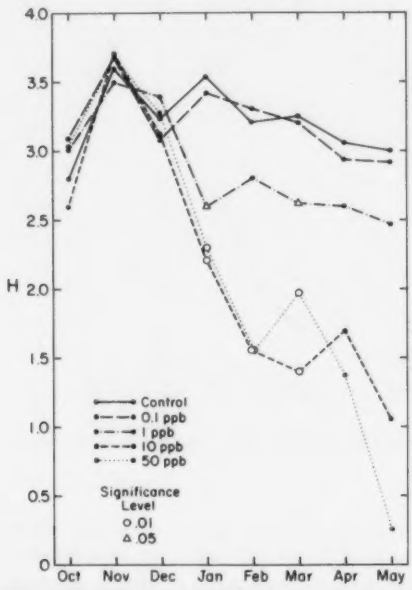


**Fig. 3.** Genera diversity (Shannon's Index) of the total insect fauna for each treatment level as a function of time

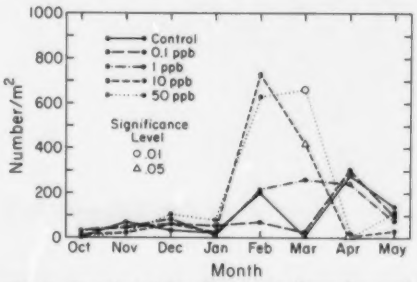


**Fig. 5.** Density of the oligochaete fauna for each treatment level as a function of time

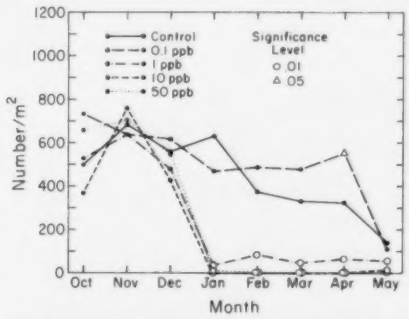


**Fig. 6.** Density of the mayfly fauna for each treatment level as a function of time

with the addition of 1 $\mu$g/L or more of diflubenzuron. Diatoms were the major algal group in all treatment levels, comprising greater than 80% of the total. The common species in order of their relative abundances were *Achnanthes lanceolata, Navicula sp., Cocconeis placentula, Nitzschia sp., Gomphonema acuminatum var coronata, and Rhoicosphenia curvata.* Addition of Dimilin® increased species richness due to the maintenance of these common species and the early establishment of *Melosira varians* and *Synedra sp.* (Figure 13), with statistical significance during the first three treatment months.

The fungal component of the stream communities experienced only temporary effects in biomass and species composition, associated with the addition of diflubenzuron. The abundance of fungal hyphae was significantly elevated initially in all the treatment concentrations (Figure 14). These differences disappeared after the first month of Dimilin® addition. The fungal species diversity was significantly depressed in all treatment concentrations (Figure 15). The differences disappeared after the first two months of Dimilin® addition. These alter-

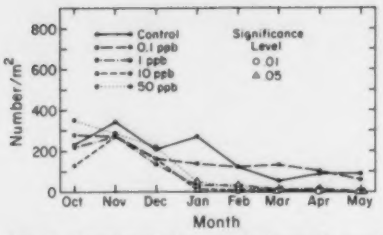


**Fig. 7.** Density of the stonefly fauna for each treatment level as a function of time

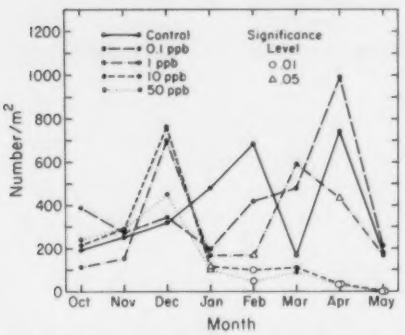


**Fig. 8.** Density of the chironomid fauna for each treatment level as a function of time

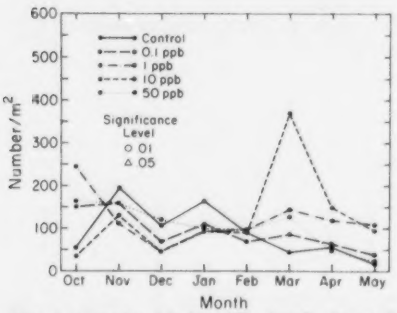


**Fig. 9.** Density of the riffle beetle fauna for each treatment level as a function of time

ations in diversity were due to reduced abundances of rare species. Despite these reductions, the fungal composition of the treatment and control streams remained qualitatively similar; *Heliscus stellatus*, *Articulospora tetracladia*, and *Tetrachaetum elegantus* were the most abundant species in all streams.

The density and community structure of the bacterial component of the stream communities were only slightly affected by the addition of Dimilin.® The density of bacteria was not affected by any treatment concentration (Figure 16). The underlying community structure was similarly unaffected until the last month of treatment, when qualitative changes were observed. Throughout the period,

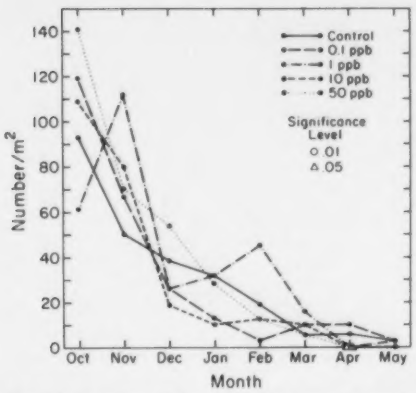


**Fig. 10.** Density of the caddisfly fauna for each treatment level as a function of time

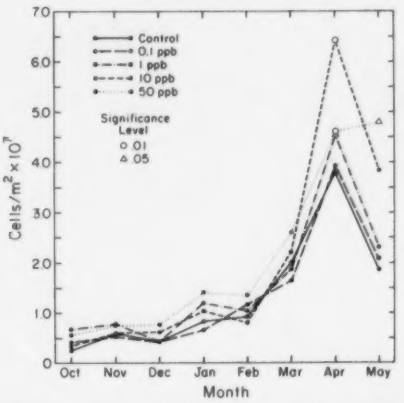


**Fig. 11.** Density of algal cells for each treatment level as a function of time

the most abundant genera in all streams were *Pseudomonas* and *Flavobacteria; Acinetobacter*, *Aeromoas*, and *Azotobacter* were common, but less abundant. During the last month of treatment, the streams receiving 1 μg/L or more of Dimilin® experienced decreased diversity (Figure 17), associated with increased relative abundances of *Pseudomonas* and corresponding decreased *Flavobacteria* and *Acinetobacter* abundances.

Water quality characteristics over the course of the study are presented in Table 2. Since there were no statistically significant differences between any of the stream channels, the water chemistry parameters are presented as monthly mean values, averaged over all streams.

## Discussion

In the stream communities, diflubenzuron acted as a selective insect control agent. It was highly toxic

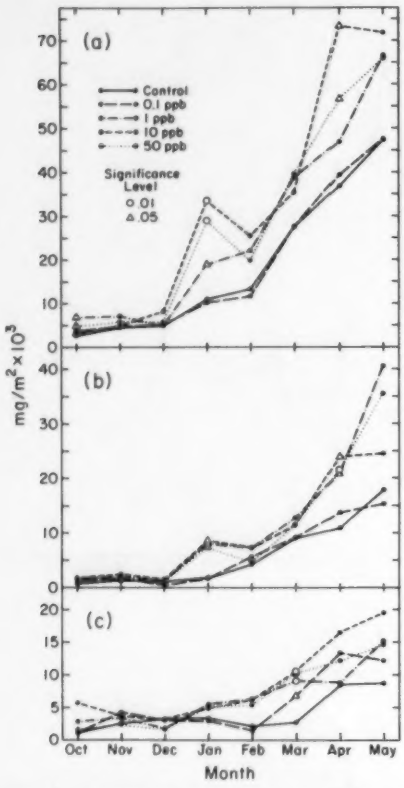


**Fig. 12.** Concentrations of (a) chlorophyll a, (b) chlorophyll c, and (c) phaeophytin for each treatment level as a function of time

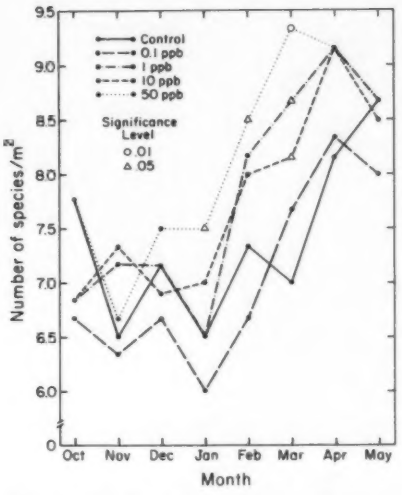


**Fig. 13.** Species richness (S-1) of diatoms for each treatment level as a function of time

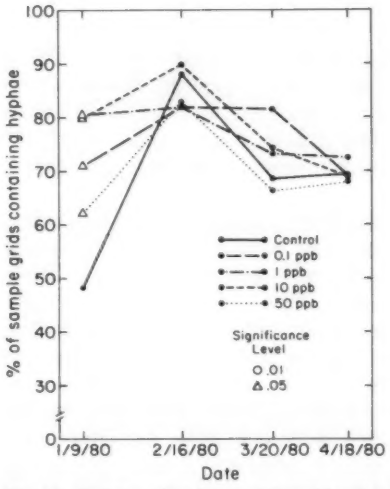


**Fig. 14.** Relative abundance of fungal hyphae for each treatment level as a function of time

to segments of the insect fauna, but relatively innocuous to the rest of the biological community. Many insect populations were sharply reduced or eliminated. Differences in the sensitivities of the various insect orders were apparent; however, the overall impact on the insect fauna was high. The fungal and algal components of the system were mildly and temporarily affected; experiencing elevated biomasses and slight shifts in relative species abundances.

The effects experienced by the algal component of the stream communities were apparently indirect, resulting from the reduced invertebrate fauna rather than direct toxicity. The increase in algal biomass coincided with large reductions in the populations of the major stream herbivores (i.e., species of *Baetis, Epeorus, Rithrogena, Paraleptophlebia, Ephemerella,* and *Orthocladiinae*) (Merritt and Cummins 1978). The shift in diatom species composition was to higher abundances of *Melosira varians* and *Synedra sp.* These diatoms have been shown to be competitively superior under conditions of space limitation (Patrick 1977). The reduction in herbivore density resulted in higher algal densities and, consequently, increased competition for scarcer attachment sites. Under such conditions, the *Melosira* and *Synedra* species would be expected to increase; as observed in this study.

The effects experienced by the fungal component were also indirect. The increase in fungal hyphae biomass coincided with large reductions in the populations of the major invertebrate leaf shredders (*Paraleptophlebia, Ephemerella, Peltoperla,* and

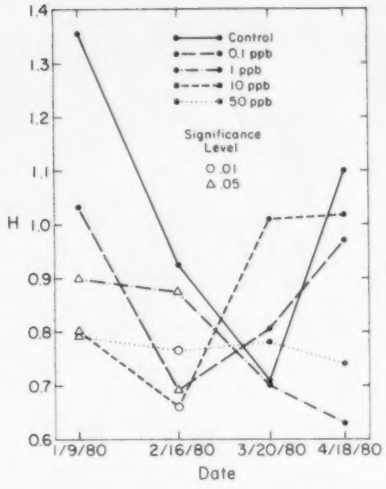


**Fig. 15.** Species diversity (Shannon's Index) of fungal spores for each treatment level as a function of time

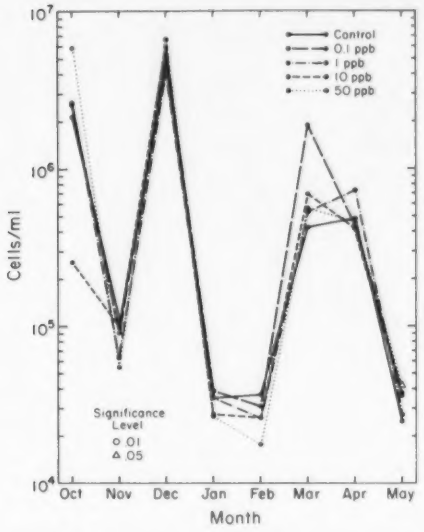


**Fig. 16.** Density of the bacterial flora for each treatment level as a function of time

*Nemoura*) (Merritt and Cummins 1978). These species shred leaf packs, ingesting the nutritious attached fungal and bacterial flora. The reduction of this insect functional group would retard the shredding process, resulting in the observed elevated fungal biomass.

The impact of diflubenzuron on some non-target insect populations may be more severe and longer lasting than on targeted pest populations in these stream communities. This is a consequence of differences between insect orders in their sensitivities to diflubenzuron and in their generation times. In aquatic systems, diflubenzuron is commonly used to control dipteran populations. Results from this study indicate that the control of the dipteran family Chironomidae would require a 10 $\mu$g/L treatment. This concentration would not only control the target populations, but would also severely reduce and/or eliminate the more sensitive mayflies and stoneflies. Due to differences in their generation times, the dipterans could recover in one to two months, whereas the mayflies and stoneflies would require a one- to two-year recovery period. Therefore, the dipteran populations would again reach pest densities, while the other more sensitive non-target species would continue to suffer long-term reductions with potential indirect effects throughout the community.

This study, therefore, supports the hypothesis that the direct toxic effects of diflubenzuron are limited to species within the phylum Arthropoda, and this specificity makes diflubenzuron and related compounds more promising insect pest control agents than the more broadly acting and more highly bioconcentrating insecticides currently in use. However, the toxicity is not specific to pest populations and the ramifications of non-target damage must be considered in assessing the benefit gained from its use.

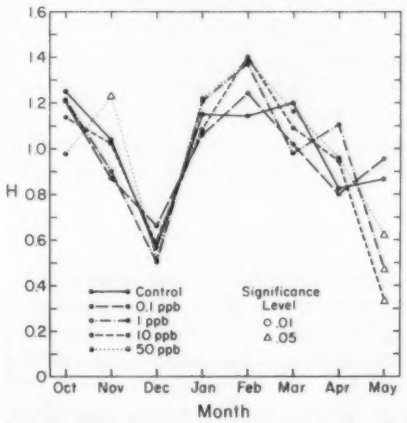


**Fig. 17.** Genera diversity (Shannon's Index) of the bacterial flora for each treatment level as a function of time

**Table 2.** Water quality characteristics—monthly averages ± s.d.

| Month | T (°C) | pH | D.O. (mg/L) | Hardness (mg/L as $CaCo_3$ equiv) | Alkalinity (mg/L as $CaCO_3$) | $NO_3$ $NO_2^-$ (mg/L) | $NH_3$ (mg/L) | Total N (mg/L) | Total $PO_4^=$ (mg/L) | o-$PO_4^=$ (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| Sept. | 14.2 | 7.0 | 9.9 | 28.5 | 2.4 | .148 | .005 | .057 | .111 | .051 |
| | ±1.0 | ±0.2 | ±0.2 | ±9.0 | ±0.1 | ±.011 | ±.003 | ±.047 | ±.015 | ±.002 |
| Oct. | 13.0 | 7.0 | 10.3 | 26.0 | 2.5 | .080 | .007 | .040 | .087 | .030 |
| | ±1.3 | ±0.1 | ±0.2 | ±3.8 | ±0.1 | ±.019 | ±.004 | ±.037 | ±.005 | ±.006 |
| Nov. | 12.8 | 6.9 | 10.5 | 25.3 | 2.3 | | | | | |
| | ±1.7 | ±0.1 | ±0.6 | ±5.6 | ±0.2 | | | | | |
| Dec. | 11.2 | 7.2 | 11.2 | 39.0 | 3.2 | .359 | .096 | .016 | .074 | .029 |
| | ±1.2 | ±0.2 | ±1.2 | ±15.1 | ±1.0 | ±.024 | ±.015 | ±.026 | ±.094 | ±.005 |
| Jan. | 10.2 | 7.1 | 11.3 | 48.5 | 3.5 | .066 | .030 | .115 | .085 | .036 |
| | ±0.9 | ±0.1 | ±0.4 | ±23.0 | ±1.6 | ±.007 | ±.013 | ±.025 | ±.012 | ±.003 |
| Feb. | 10.8 | 7.1 | 10.9 | 57.5 | 4.6 | | | | | |
| | ±0.6 | ±0.1 | ±0.3 | ±4.4 | ±0.4 | | | | | |
| Mar. | 11.2 | 7.2 | 10.8 | 60.8 | 4.9 | .510 | .028 | .234 | .081 | .014 |
| | ±0.8 | ±0.0 | ±0.3 | ±19.7 | ±0.5 | ±.180 | ±.033 | ±.261 | ±.185 | ±.002 |
| Apr. | 12.0 | 7.2 | 10.5 | 41.8 | 3.6 | .503 | .014 | .131 | .035 | .027 |
| | ±0.9 | 0.0 | ±0.2 | ±6.0 | ±0.4 | ±.008 | ±.017 | ±.056 | ±.010 | ±.007 |

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## References

Ali, A., and M. S. Mulla: Chemical control of nuisance midges in the Santa Ana River Basin, Soulhern California. J. Econ. Entomol. **70,** 191 (1977a).

———: The IGR dibenzuron and organophosphorus insecticides against nuisance midges in man-made residential-recreational lakes. J. Econ. Entomol. **70,** 571 (1977b).

———: Effects of chironomid larvicides and diflubenzuron on non-target invertebrates in residential-recreational lakes. Env. Entomol. **7,** 21 (1978a).

———: Impact of the insect growth regulator diflubenzuron on invertebrates in residential-recreational lakes. Arch. Env. Contam. Toxicol. **7,** 483 (1978b).

Buchanan, F. E., and N. E. Gibbons: Bergey's manual of determinative bacteriology, 8 ed. Williams and Wilkins: Baltimore, MD (1974).

Colwell, A. E., and C. H. Schaeffer: Diets of *Ictalurus nebulosus* and *Pomoxis nigromaculatus* altered by diflubenzuron. Can. J. Fish. Aquat. Sci. **37,** 632 (1980).

Hajjar, N. P., and J. E. Casida: Insecticidal benzoylphenyl ureas: Structure–activity relationships on chitin synthesis inhibitors. Science **200,** 1499 (1978).

Hustedt, F: Dia Suswasser-flora mitteleuropas. Jena Verlag von Gustau Fischer: Germany (1930).

Ingold, C. T.: Aquatic Hyphomycetes of decaying alder leaves. Trans. Brit. Mycol. Soc. **25,** 339 (1942).

Ingold, C. T., and V. J. Cox: *Heliscus stellatus sp. nov.* an aquatic Hyphomycetes. Trans. Brit. Mycel. Soc. **40,** 155 (1957).

Ivie, G. W., and J. E. Wright: Fate of diflubenzuron in the stable fly and house fly. J. Agr. Fd. Chem. **26,** 90 (1978).

Ivie, G. W., D. L. Bull, and J. A. Veech: Fate of diflubenzuron ir /ater. J. Agr. Fd. Chem. **28,** 330 (1980).

Julin, A. M., and H. O. Sanders: Toxicity of the IGR, diflubenzuron, to freshwater invertebrates and fishes. Mosquito News **38,** 256 (1978).

Lacey, L. A., and M. S. Mulla: Larvicidal and ovicidal activity of Dimilin® against *Simulium vittatum*. J. Econ. Ent. **70,** 369 (1977).

———: Factors affecting the activity of diflubenzuron against *Simulium* larvae (*Diptera: Simuliidae*). Mosquito News **38,** 264 (1978a).

———: Biological activity of diflubenzuron and three new IGR's against *Simulium vittatum* (*Diptera: Simuliidae*). Mosquito News **38,** 377 (1978b).

McKague, A. B., and R. B. Pridmore: Toxicity of Altoside® and Dimilin® to juvenile rainbow trout and coho salmon. Bull. Environ. Contam. Toxicol. **20,** 167 (1978).

Meola, S. M., and R. T. Mayer: Inhibition of cellular proliferation of imaginal epidermal cells by diflubenzuron in pupae of the stable fly. Science **207,** 985 (1980).

Merritt, R. W., and K. W. Cummins: An introduction to the aquatic insects of North America. Kendall/Hunt: Dubuque, IA. (1978).

Metcalf, R. L., Po-Yung Lu, and S. Bowlus: Degradation and environment fate of 1-(2,6-difluorobenzyoyl)-d(-4chlorophenyl) urea. J. Agr. Fd. Chem. **23,** 359 (1975).

Miller, R. W.: TH 6040 as a feed additive for control of the face fly and house fly. J. Econ. Entomol. **67,** 697 (1974).

Miller, R. W., C. Corley, and K. R. Hill: Feeding TH 6040 to chickens: Effect on larval house flies in manure and determination of residue in eggs. J. Econ. Entomol. **68,** 181 (1974).

Miura, T., and R. M. Takahaski: Effects of the IGR, TH 6040, on nontarget organisms when utilized as a mosquito control agent. Mosquito News **35,** 154 (1975).

Moore, R. F., and H. M. Tuft: Boll Weevils: Chemo-sterilization of both sexes with Busulfan plus Thompson-Hayward TH 6040. J. Econ. Entomol. **68,** 96 (1975).

Mulla, M. S., and H. A. Darwazeh: Effects of the insect growth regulator Dimilin® or TH 6040 on mosquitoes and some non-target organisms. Mosquito News **35,** 211 (1975).

———: The IGR Dimilin® and its formulations against mosquitoes J. Econ. Entomol. **69,** 309 (1976).

Mulla, M. S., H. A. Darwazeh, and R. L. Norland: Insect growth regulators: Evaluation procedures and activity against mosquitoes. J. Econ. Entomol. **67,** 329 (1974).

Mulla, M. S., W. L. Kramer, and D. R. Barnard: Insect growth regulators for control of chironomid midges and residential-recreation lakes. J. Econ. Entomol. **69,** 285 (1976).

Nimmo, D. R., T. L. Hamaker, J. C. Moore, and C. A. Sommers: Effect of diflubenzuron on an estuarine crustacean. Bull. Environ. Contam. Toxicol. **22,** 767 (1979).

Nimmo, D. R., T. L. Hamaker, J. C. Moore, and R. A. Wood: Acute and chronic effects of Dimilin® on survival and reproduction of *Mysidopisis bahia*. In J. G. Eaton, P. R. Parrish, and A. C. Hendricks, (eds.): Aquatic toxicology, ASTM STP 707, American Society for Testing and Materials (1980).

Patrick, R: Ecology of freshwater diatoms and diatom communities. In Dietrich Warner (ed): The biology of diatoms, Botanical Monographs, Vol. 13, p. 234. Blackwell Scientific Publications, Oxford, England (1977).

Patrick, R., and C. W. Reimer: The diatoms of the United States, Vol I. Monographic series of Academy of Natural Sciences of Philadelphia, No. 13. Philadelphia, PA (1966).

———: The diatoms of the United States, Vol. II. Monographic series of Academy of Natural Sciences of Philadelphia, No. 13. Philadelphia, PA (1975).

Petersen, R. H.: Aquatic Hyphomycetes from North America I. Aleurio-sporae (Part I), and key to the genera. Mycologia **54,** 117 (1962).

———: Aquatic Hyphomycetes from North America II. Aleurio-sporae (Part II), and Blastosporae Mycologia **55,** 18 (1963a).

———: Aquatic Hyphomycetes from North America. III. Phialosporae and miscellaneous species. Mycologia **55,** 570 (1963b).

Post, L. C., B. J. DeJong, and W. R. Vincent: 1-(2,6-disubstituted benzyol)-3-phenylurea insecticides: Inhibitors of chitin synthesis. Pest. Biochem. **4,** 473 (1974).

Pritchard, P. H., and A. W. Bourquin: Fate of Dimilin® in laboratory estuarine ecosystems. In U.S.E.P.A. Report. Gulf Breeze Environmental Research Laboratory, Gulf Breeze, FL (1979).

Ranzoni, F. V.: The aquatic Hyphomycetes of California. Farlowia **4,** 353 (1953).

Ruzo, L., M. J. Zabik, and R. D. Schuetz: Photochemistry of bioactive compounds, 1-(4-chlorophenyl)-3-(2,6 dichlorobenzoyl) ureas. J. Agr. Fd. Chem. **22,** 1106 (1974).

Samuelson, D. F.: Water Quality: Western Fish Toxicology Station and Western Oregon Rivers. Ecological Research Series, U.S. Environmental Protection Agency, EPA-600/3-76-077 (1976).

Schaeffer, C. H., and E. F. Dupras: Factors affecting the stability of Dimilin® in water and the persistence of Dimilin® in field waters. J. Agr. Fd. Chem. **24,** 733 (1976).

———: Residues of diflubenzuron (1-(4-chlorophenyl)-3-(2,6 difluorobenzoyl)urea in pasture soil, vegetation and water following aerial application. J. Agr. Fd. Chem. **25,** 1026 (1977).

Schaeffer, C. H., E. F. Dupras, R. J. Stewart, L. W. Davidson, and A. E. Colwell: The accumulation and elimination of diflubenzuron by fish. Bull. Env. Contam. Toxicol. **21,** 249 (1979).

Shayegani, M., A. M. Lee, and L. M. Parson: A scheme for the identification of nonfermentative gram-negative bacteria. Health Lab. Science **14,** 83 (1976).

Steele, M. B.: Identification of nonfermentative gram-negative Bacilli by conventional biochemicals and a dichotomous scheme. Wilmington Medical Center, Delaware Division-Microbiology, Wilmington, DE (1979).

Steelman, C. D., J. E. Farlow, T. P. Breaud, and P. E. Schilling: Effects of growth regulators on *Psorophore columbiae* (Dyar and Knab) and non-target aquatic insect species in rice fields. Mosquito News **35,** 67 (1975).

Weber, C. I.: Biological field and laboratory methods for measuring the quality of surface waters and effluents. U.S. Environmental Protection Agency Publication, EPA-670/4-73001, Cincinnati, OH (1973).

Williams, D. A.: The comparison of several dose levels with a zero dose control. Biometrics **28,** 519 (1972).

Wright, F. E., and R. L. Harris: Ovicidal activity of Thompson-Hayward TH-6040 in the stable fly and horn fly after surface contact of adults. J. Econ. Entomol. **69,** 728 (1976).

Wright, J. E., and G. E. Spates: 1976. Reproductive inhibition activity of the insect growth regulator TH-6040 against the stable fly and the house fly: Effects on hatchability. J. Econ. Entomol. **69,** 365 (1976).

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